Hematological Evaluation of Primary Extra Nodal Versus Nodal NHL: A Study from North India

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Abstract Primary extra nodal lymphomas (EN-NHL) are different from primary nodal non-Hodgkin's lymphoma (N-NHL) and are comparatively less common. Hemogram findings and bone marrow involvement is less studied and very few reports are available in the literature. The present study is a retrospective analysis of bone marrow samples evaluated for staging of non-Hodgkin's lymphoma. The age, sex distribution, clinical features, and site of presentation, hemogram findings, pattern of bone marrow involvement and grade of reticulin fibrosis was noted. These findings were compared with the type of non-Hodgkin's lymphoma and prognostic information was determined. A total of 647 cases of NHL, which underwent bone marrow examination for staging, over a seven year period, were retrieved and analyzed for all hematological parameters. Prevalence of EN-NHL was 23.5% (152/647), while nodal NHL comprised 76.5% (495/647) of all NHL cases. 90.1% (137/152) cases of EN-NHL were adult patients, out of which 15.3% (21/137) cases showed bone marrow infiltration as compared to 89% (441/495) adult primary nodal NHL cases, of which 39% (175/441) showed bone marrow infiltration. 9.9% (15/152) cases of EN-NHL were pediatric patients, out of which 40% (6/15) showed

bone marrow infiltration, while 10.9% (54/495) of nodal NHL cases were pediatric, of which 20.3% (11/54) showed bone marrow infiltration. Hemogram findings were not found useful in predicting bone marrow infiltration in both nodal as well as EN-NHL. 100% (6/6) of pediatric patients had high grade lymphoma as compared to 48% (9/21) of adult patients, showing bone marrow infiltration in EN-NHL group. Reticulin fibrosis also did not reveal relation with grading of NHL. Prognostically EN-NHL of stomach and central nervous system were found to be better than EN-NHL of other sites, as none of these cases showed bone marrow infiltration. EN-NHL can involve various sites and the prognosis depends upon the sites of disease as well as the type of NHL. Moreover, pediatric EN-NHL cases are likely to have poorer prognosis, due to increased risk of bone marrow involvement as compared to their counterparts having primary nodal NHL. Bone marrow infiltration at times cannot be assessed reliably from hemogram findings only and a bone marrow biopsy for staging is mandatory.

Keywords Bone marrow · Extranodal non-Hodgkin's lymphoma · Hemogram · Reticulin

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Introduction

Primary extra nodal lymphoma (EN-NHL) are a heterogeneous category of tumors that are considered to be different from primary nodal non-Hodgkin's lymphoma and are less common than primary nodal NHL (N-NHL) [1]. The clinical implication of this difference is not very clear as knowledge of extra nodal lymphomas as a separate group is limited. Studies imply that the prognosis is dependent on the stage and histology rather than the primary localization

per se [2]. There is a paucity of data regarding bone marrow infiltration in primary EN-NHL. The present study was undertaken to evaluate the incidence of EN-NHL as compared to nodal NHL and also to analyze various sites of extra nodal NHL and their correlation with age, sex and pattern of bone marrow infiltration. We also evaluated the peripheral blood findings keeping in mind whether the bone marrow showed infiltration or not.

Materials and Methods

This is a retrospective analysis of 647 cases of non Hodgkin's lymphoma sent to the department of Hematology for staging bone marrow over a 7 year period. The study is based on morphology and immunohistochemistry is not part of the objectives, which had been carried out only in a small number of cases and therefore not included in the analysis. We feel that immunohistochemistry requires to be done in the primary tumor tissue rather than the bone marrow for sub classifying.

All the cases of bone marrow aspiration and trephine biopsies done for primary staging of NHL were retrieved from the archival material. Bone marrow aspiration was performed from posterior–superior iliac spine followed by bilateral trephine biopsies, in each case. All the aspiration slides were stained with May-Grunwald Giemsa stain and all the trephine biopsies were stained with haematoxylin and eosin stain and reticulin stain. Cases of primary splenic NHL, patients on chemotherapy and recurrence of NHL were excluded. Cases of NHLs with unknown primary site and cases of lymphomas showing leukemic conversion were also excluded from the present study. Clinical details of all the cases were reviewed for age, sex and clinical diagnosis. Hemogram, findings along with peripheral blood film and bone marrow samples were also reviewed.

Statistical Analysis

Appropriate statistical analysis was carried out using the SPSS version 12 for data analysis.

Results

This study included a total of 647 cases of NHL, who underwent evaluation for bone marrow infiltration, over a 7 year period. International working formulation was followed to classify NHL into low, intermediate and high grade. Out of total 647 cases, there were 495 (76.5%) cases of primary nodal NHL and 152 (23.5%) cases of extra nodal NHL. A total of 213 (33%) cases out of 647 cases

showed bone marrow infiltration. 186/495 cases (37.5%) of nodal NHL and 27/152 cases (17.7%) of extra nodal NHL showed bone marrow infiltration.

Prevalence of EN-NHL was 23.5% of all NHL and age in adult patients ranged from 13 to 81 years with a male to female ratio of 1.8:1. A total of 441/495 (89.1%) cases of primary nodal NHL were adult patients and 175/441 cases (39.6%) showed bone marrow infiltration. Similarly, a total of 137/152 (90.13%) cases of EN-NHL were adult patients, out of which 21(15.32%) cases showed bone marrow infiltration.

In pediatric patients, the age ranged from 1 to 12 years with male to female ratio of 4:1. There was no infant in this study group. A total of 54/495 (10.9%) cases of primary nodal NHL were pediatric patient. 11/54 cases (20.3%) showed bone marrow infiltration and 43/54 cases (79.6%) did not show marrow infiltration. Similarly, a total of 15/152 (9.8%) cases of EN-NHL were pediatric patients and 6/15 cases (40%) showed bone marrow infiltration. Abnormal hemogram findings with bone marrow infiltration was noted in 46.8% of adult primary nodal NHL cases and 54.5% of pediatric nodal NHL cases as compared to 52.3% of adult EN-NHL cases and 50% of pediatric EN-NHL cases. These findings are highlighted in detail in Table 1.

In the group of nodal NHL without bone marrow infiltration showing abnormal hemogram findings, 40 cases had only anemia, 20 cases had leucocytosis, 22 cases had thrombocytosis, 6 patients had thrombocytopenia, 2 had pancytopenia and 6 cases had bicytopenia. 20 cases were pediatric patients and 76 were adult cases. In the group of nodal NHL with infiltration into the bone marrow, showing abnormal hemogram, 6 were pediatric cases and 82 were adult cases. 40 patients had anemia, 20 had leucocytosis, 3 had thrombocytosis, 5 had thrombocytopenia and 20 cases had bicytopenia. Shift to left was noted in 20 cases and peripheral blood film showed atypical cells in 6 cases.

In the group of extra nodal NHL without bone marrow infiltration showing abnormal hemogram findings, 11 cases had only anemia, 4 cases had thrombocytosis and 2 patients had thrombocytopenia and 8 cases had bicytopenia. In the group of extra nodal NHL with infiltration into the bone marrow showing abnormal hemogram, 6 patients had anemia, 3 had leucocytosis, 3 had thrombocytosis and 2 case had bicytopenia. Shift to left was noted in 2 cases. On statistical analysis, it was found that hemogram findings were not useful in predicting bone marrow infiltration (P -value > 0.5). Pattern of bone marrow infiltration was predominantly diffuse in pediatric N-NHL (63.6%) and EN-NHL (83.3%) cases, while in adult cases it was predominantly nodular in N-NHL (34.2%) and diffuse in EN-NHL (47.6%) (see Table 2). Bone marrow necrosis was noted in 19.23% of cases of bone marrow infiltration in

Table 1 Distribution of nodal and extra nodal NHL cases with regards to number of cases, abnormal hemogram findings and bone marrow infiltration

Parameters	Adult cases (%)	Pediatric cases (%)
Nodal-NHL	441/495 (89.1)	54/495 (10.9)
Extranodal-NHL	137/152 (90.1)	15/152 (9.9)
Bone marrow infiltration (nodal-NHL)	175/441 (39.6)	11/54 (20.3)
Bone marrow infiltration (extranodal-NHL)	21/137 (15.3)	6/15 (40)
Abnormal hemogram in nodal-NHL cases with infiltration	82/175 (46.8)	6/11 (54.5)
Abnormal hemogram in extranodal-NHL cases with infiltration	11/21 (52.3)	3/6 (50)

Table 2 Pattern of infiltration in adult and pediatric nodal and extranodal NHL cases

Pattern of infiltration	Adult nodal (%)	Adult extranodal (%)	Pediatric nodal (%)	Pediatric extranodal (%)
Diffuse	50/175 (28.5)	10/21 (47.6)	7/11 (63.6)	5/6 (83.3)
Nodular	60/175 (34.2)	6/21 (28.5)	1/11 (9)	0/6 (0)
Paratrabeular	12/175 (6.8)	1/21 (4.7)	0/11 (0)	0/6 (0)
Interstitial	27/175 (15.4)	1/21 (4.7)	1/11 (9)	1/6 (16.6)
Mixed	26/175 (14.8)	3/21 (14.2)	2/11 (18.2)	0/6 (0)

extra nodal NHL. Diffuse reticulin fibrosis (3+) was seen in 30% of pediatric cases as compared to 25% of adult cases.

Extra nodal non-Hodgkin's lymphoma was seen to involve different sites as stomach 40(0) in adults and 2(0) in pediatric cases, intestine 25(4) (e.g. see Fig. 1) in adults and 9(4) in pediatric cases, Para spinal region 6(4) in adults and 1(1) in pediatric cases, soft tissue 6(1) in adults and 1(1) in pediatric cases, central nervous system 11(0) in adults and 1(0) in pediatric cases, orbit 5(2) in adults and 1(0) in pediatric cases. Other sites in adult patients included tonsil 5(1), testis 7(1), pharynx 5(1), localized bone 5(1), skin 4(1) (e.g. see Fig. 2), nasal cavity 4(0), Para nasal sinuses 3(0), thyroid 3(1), salivary gland 2(0), breast 2(0) and one case each in kidney (1), gallbladder (1), soft palate (1), lacrimal gland (1). The figures given in brackets show number of cases showing bone marrow infiltration. Immunohistochemistry (IHC) was performed on only those bone marrow biopsy samples in which the infiltrate was either patchy or interstitial in nature or in cases having extra nodal lymphoma at rare sites, to confirm the diagnosis. The markers used were predominantly CD-20 as B-cell marker and CD-3 as T-cell marker (see Fig. 2). Extra nodal NHL was seen most commonly in gastrointestinal tract, para spinal region, soft tissue and central nervous system. Testicular EN-NHL was seen more common in elderly patients with age more than 60 years (71.4%). EN-NHL in tonsil, stomach and central nervous system are more commonly in age groups of 45–60 years. Para-spinal EN-NHL showed a more aggressive behavior as compared to EN-NHL in stomach and central nervous

system. Cases of both adult and pediatric EN-NHL and N-NHL showing bone marrow infiltration were assessed for grade of NHL and reticulin fibrosis in the bone marrow and are the findings are highlighted in Table 3. It was observed that 100% of pediatric patients had high-grade lymphoma as compared 48% of adult patients showing bone marrow infiltration in extra nodal NHL. 15% of adult EN-NHL cases had intermediate grade NHL and rest 37% cases were of low grade NHL. 24% adult EN-NHL cases showing bone marrow infiltration had 1+ reticulin, 38% had 2+ and 38% cases had 3+ reticulin fibrosis. Similarly, 33.3% of pediatric EN-NHL cases had 1+ reticulin, 16.7% had 2+ and 50% cases had 3+ reticulin fibrosis in the marrow. Reticulin fibrosis did not reveal any co-relation with grading of NHL.

Discussion

Primary extra nodal lymphomas (EN-NHL) are a heterogeneous category of tumors that are considered different and are less common than primary nodal NHL (N-NHL) [1]. The knowledge of EN-NHL as a separate category is limited. The most common sites included in this category are gastrointestinal tract and nasopharynx, the other sites being thyroid, breast, salivary gland, lung, brain, testis, orbit and skin. There is paucity of data regarding bone marrow infiltration in EN-NHL. One of the interesting concepts in extra nodal lymphomas is lymphomas arising in mucosa associated lymphoid tissue, also known as MALToma, a term proposed by Isaacson and Wright in

Fig. 1 A case of low grade lymphoma of small intestine in an adult female showing paratrabeular infiltrate of lymphoma cells, rimmed by an eosinophil rich infiltrate

Fig. 2 High power photomicrograph (400×) showing two nodular infiltrates of lymphoma cells which are positive for CD 20 in a case of high-grade B-cell lymphoma of thyroid

1983 [3, 4]. These arise most commonly in gastrointestinal tract and other sites being respiratory tract, ocular adnexa, salivary gland, thyroid, breast, urinary bladder, kidney, prostate, liver, gallbladder, uterine cervix, thymus and skin [5]. Krol et al. [11], compared nodal and extra nodal NHL patients to determine differences in characteristics at diagnosis, response to treatment, patterns of failure and survival. Stage I EN-NHL patients as a group had a poor prognosis than N-NHL patients. Among EN-NHL patients, considerable variations in response, survival and relapse rates were observed, with gastric NHL patients having the

best and central nervous system NHL patients having the worst prognosis. In present study, none of the gastric and central nervous system NHL patients had bone marrow infiltration, thus implying that EN-NHL from these sites have a relatively better prognosis in our population group.

Marrow involvement also varies according to histological subtypes with 70–80% in small lymphocytic lymphoma, 60–70% in mantle cell lymphoma, 40–60% in follicular centre cell lymphoma, predominantly composed of small cells and 25–35% in follicular centre cell lymphoma, predominantly composed of large cells or the blastic subtype [6]. Significant cytopenias are rare unless marrow involvement is extensive. 40–70% of patients with low-grade lymphomas (small lymphocytic lymphoma and small-cleaved follicular centre cell lymphoma) have peripheral blood involvement that often does not have a major impact on survival. Less than 10% of the patients with the higher-grade histological patterns present with a leukemic phase that is associated with a poor prognosis [7].

The most common extra nodal site of involvement is stomach, which was also seen in present study. Gastric maltoma represents approximately two-thirds of all Maltoma and up to one-half of primary gastric lymphoma. Intestinal lymphomas originating in the small bowel are more common than those arising in the large bowel [8]. Immunoproliferative small intestinal disease (IPSID) can also be considered as a subtype of B-cell maltoma [9]. Lymphomas of the nasal cavity and para nasal sinuses are also more advanced and have an increased risk for CNS involvement. Other rare extra nodal lymphomas include salivary gland, thyroid, pulmonary and orbital lymphomas; all have unique presentations and histopathological features and may be associated with autoimmune disorders.

Most of salivary gland lymphomas are of low grade and localized [10]. Most of the thyroid lymphomas have localized stage IE or IIE disease and the prognosis worsens with penetration of the thyroid capsule, tumor bulk, advanced stage and intermediate to high-grade histological types [11]. In the present study, three cases of thyroid lymphoma were seen and none of these cases showed bone marrow infiltration.

Primary brain lymphomas account for less than 2% of extra nodal lymphomas, but the incidence is increasing in

Table 3 Grade of NHL and degree of reticulin fibrosis in bone marrow of adult and pediatric nodal and extranodal NHL cases with infiltration

Site	Adult cases	Pediatric cases	Grade of NHL (adult) LG/IG/HG*	Grade of NHL (pediatric) LG/IG/HG*	Marrow fibrosis [1] (adult)	Marrow fibrosis [1] (pediatric)
Nodal-NHL	175	11	105/10/60	2/1/8	1+ (24), 2+ (61), 3+ (90)	1+ (2), 2+ (4), 3+ (5)
Extranodal-NHL	21	6	9/3/9	0/0/6	1+ (4), 2+ (9), 3+ (8)	1+ (2), 2+ (1), 3+ (3)

* Low grade/intermediate grade/high grade

immunocompromised and immunodeficient hosts [12]. Majority of these are intermediate to high grade and are associated with Epstein-Barr virus. Testicular lymphoma is the most common testicular tumor beyond the age of 60 years and the most common bilateral testicular tumor [13]. The predominant histological type is diffuse large cell lymphoma, usually of B-cell origin. Primary ovarian lymphomas are rare and patients have poor prognosis with 5-year survival rate of less than 25% [14]. Primary bone lymphomas account for 3–5% of extra nodal lymphomas [15] and majority are intermediate to high-grade B-cell lymphomas. Prognosis depends on the bones involved, (with the femur the best and the spine the worst) histological type, stage and presence of soft tissue involvement [16]. Other unusual sites of lymphoma have included almost any organ and may have unique presentations.

Infiltrative bone marrow conditions like NHL are known to cause an increase in reticulin fibrosis and the grade of fibrosis usually varies with the extent of bone marrow involvement. In present study, though reticulin fibrosis did not correlate with the grade of NHL, but most of the adult and pediatric NHL cases with diffuse pattern of bone marrow infiltration had 3+ reticulin fibrosis.

Summary

In the present study, NHL, whether nodal or extra nodal, were more commonly seen in the adult age group and N-NHL was more common than EN-NHL. Pediatric NHL were usually of the high grade type, while in adult cases, N-NHL were predominantly of low grade type and EN-NHL had almost similar distribution of low and high grade subtypes. Abnormal hemogram findings failed to reveal any correlation with bone marrow infiltration in both adult and pediatric cases. Diffuse pattern of infiltration was more common in pediatric nodal and extra nodal NHL, while nodular pattern in adult N-NHL and diffuse in adult EN-NHL. Reticulin fibrosis did not reveal any statistically significant correlation with grade of NHL, though 3+ reticulin fibrosis was more commonly observed in N-NHL as compared to EN-NHL. The common sites of EN-NHL were stomach, intestine, para spinal and CNS. Stomach and CNS NHL cases formed the best prognostic group as none of the cases had evidence of bone marrow infiltration, while para spinal region NHL and pediatric intestinal NHL formed the worst prognostic group in terms of bone marrow involvement. The study also concludes that pediatric EN-NHL is more likely to involve bone marrow than pediatric N-NHL cases. Hence, a careful staging is mandatory in all cases of NHL, which includes history, physical examination, blood chemistry, CT scan and a bone

marrow biopsy. This information may be supplemented with an ear, nose and throat consultation, esophagogastroduodenoscopy, colonoscopy, endoscopic ultrasound of the stomach and cytogenetic/immunohistochemical analysis of the tumors.

Conflicts of interest None.

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